ELECTROPHORESIS

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ELECTROPHORESIS

- **MOVING BOUNDARY ELECTROPHORESIS**
- **PAPER ELECTROPHORESIS**

- Electrophoresis is a technique used to separate macromolecules in a fluid or gel based on their charge, binding affinity, and size under an electric field.
- In the year 1807, Ferdinand Frederic Reuss was the first person to observe electrophoresis.
- He was from Moscow State University. Anaphoresis is the electrophoresis of negative charge particles or anions whereas cataphoresis is electrophoresis of positive charge ions or cations.
- Electrophoresis has a wide application in separating and analysing biomolecules such as proteins, plasmids, RNA, DNA, nucleic acids.

- Electrophoresis Principle and its types:
- Charged macromolecules are placed in the electric field move towards the negative or positive pole based on their charge. Nucleic acid has a negative charge and therefore it migrates towards the anode.
- This technique is divided into two types viz slab electrophoresis and capillary electrophoresis.
- Types of Electrophoresis:

1.Capillary electrophoresis

1. **Gel electrophoresis**
2. Paper electrophoresis

2.Slab electrophoresis

1. Zone electrophoresis
2. **Immunoelectrophoresis**
3. Isoelectrofocusing

- The moving boundary method was the first used by Tiselius to demonstrate the efficacy of the electrophoretic process.
- This method allows the charged species to migrate in a free moving solution in the absence of a supporting medium.
- Samples are fractioned in a U shaped tube that has been filled with unstabilized buffer.
- An electrical field is applied by means of electrodes at the ends of the U tube.
- Separation takes place as a result of difference in mobilities.
- Apparatus consists of U tube, with electrodes located at the two ends used to apply an electric field.
- The lower part of the cell is filled with lyophillic solution under examination, sometimes the sample solution is introduced into the bottom of the U tube through a capillary arm, while upper part contains only the buffer solution.
- Care must be taken to minimize the disturbing effect of convection caused by an increase in temperature during the passage of current through the solution.
- For this purpose, the apparatus is placed in constant temperature bath at 4°c. 6

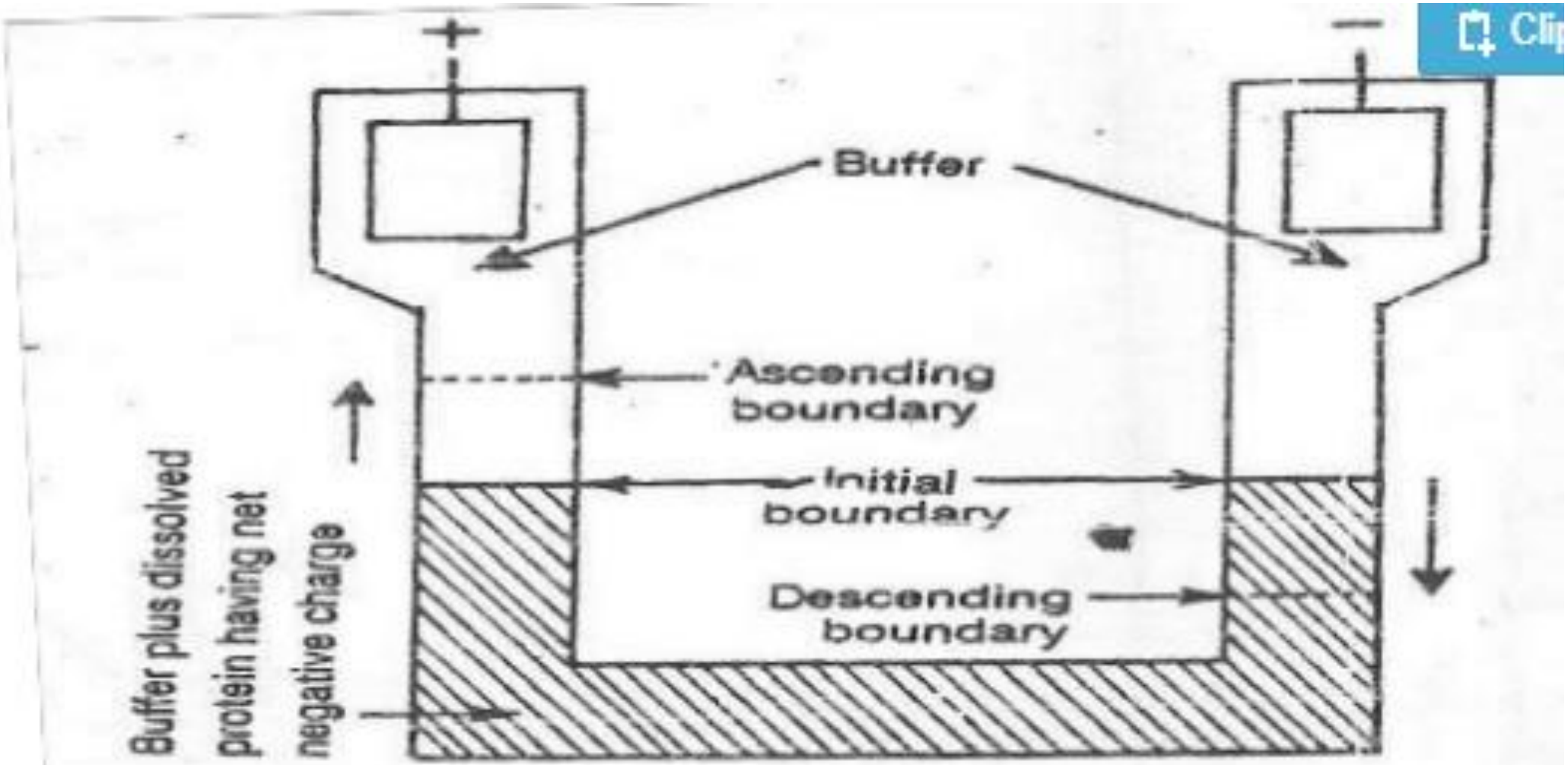

Fig. 43.1: *Schematic diagram of tiselius moving-boundary electrophoresis apparatus*

- Anionic species can be separated by using a narrow-bore tube as the separation chamber.
- It is connected with Anode and Cathode compartment Anode compartment and narrow-bore tube are filled with an electrolyte Sample is introduced into the Cathode compartment Anionic species migrate towards the anode and sample anions can never pass the anionic species of the leading electrolyte because its effective mobility is higher.
- Mobilities of the anionic species of the sample differ, however, so that some of them will migrate forward.

In Moving Boundary Electrophoresis,

- Zones generally contains more ionic species of the sample.
- Composition of the sample plays an important role in the determination of the concentrations, pH values and conductivities of the different zones.
- So Effective mobilities can be measured by using Moving boundary electrophoresis.

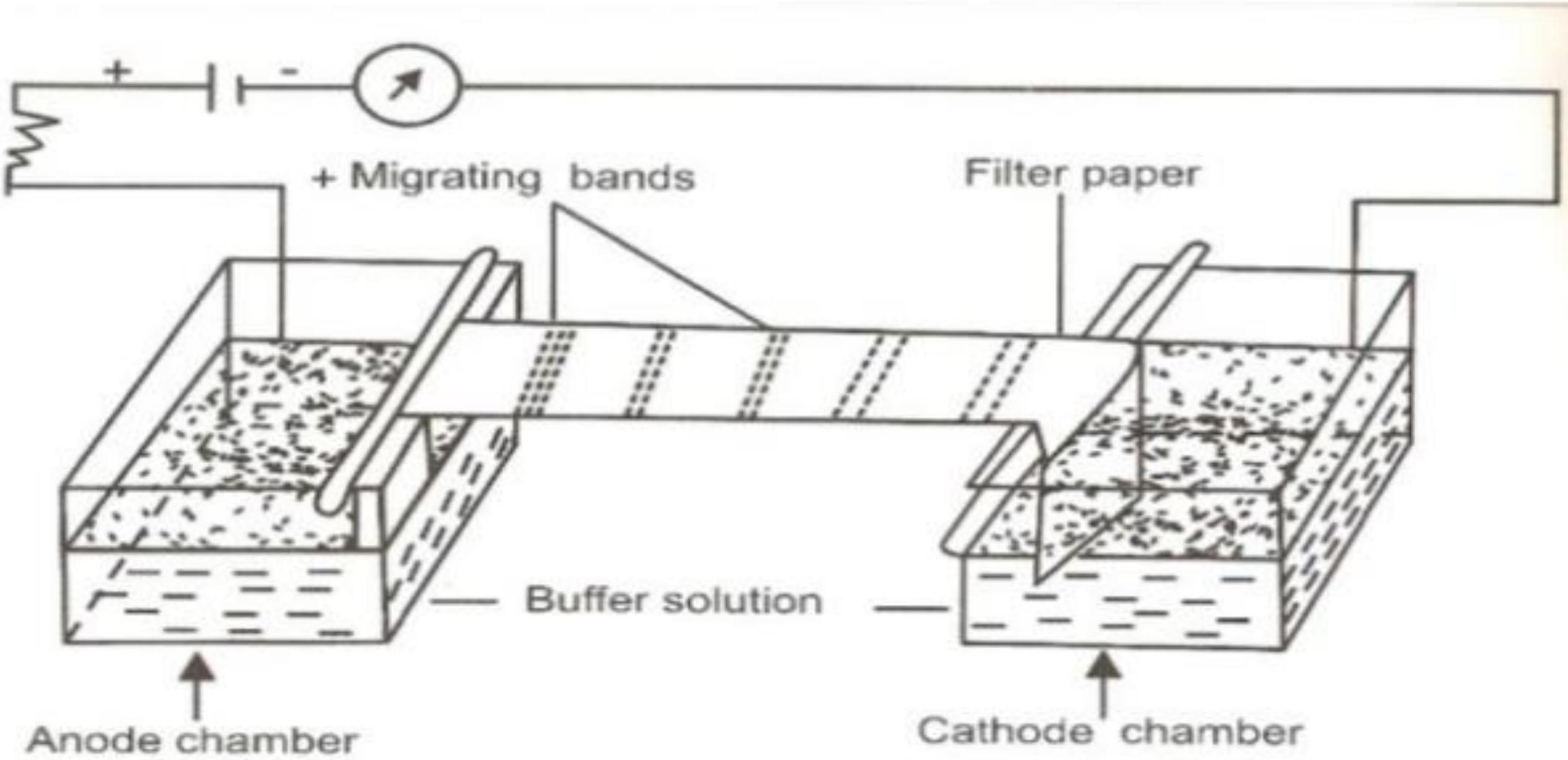

Fig. 28.1: Paper electrophoresis (one-dimensional)

- The technique of paper electrophoresis is simple and inexpensive and requires only micro quantities of plasma for separation.
- The support medium is a filter paper .
- The electrophoresis apparatus in its simplest form consists of two troughs to contain buffer solution, through which electric current is passed. Frequently used in isolating proteins, amino acids and oligopeptides.
- Procedure
- 1) A long strip of filter paper is moistened with a suitable buffer solution of the desired p H and the sample is applied transversely across the central part of the strip.
- 2) Ends are fixed to dip in buffer solutions in two troughs fitted with electrodes.
- 3)Electric field of about 20 volts/cm is established.
- 4)The charged particles of sample migrate along the strip towards respective electrodes of opposite polarity, according to net charges, sizes and interactions with the solid matrix.

- 5)Homogeneous group of particles migrate as a separate band
- 6)The electrophoresis is carried out for 16-18 hours.
- 7) Separated Proteins are fixed to a solid support using a fixative such as Acetone or Methanol
- 8)Proteins are stained (bromophenol blue) to make them visible
- 9) The separated proteins appear as distinct bands
- 10)Drawback-long time interval and blurring of margins
- The different fractions appear as blue coloured bands across the filter paper starting from the moving boundary backwards.
- If a quantitative estimation is required for each fraction, the bands may be carefully cut and eluted, or the bands may be scanned optically in a densitometer.
- In human plasma five different bands can be identified on paper electrophoresis observation.